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In re Application of: HIRABAYASHI et al.

SERIAL NO.: 09/646,135 **Group Art Unit:** 1635

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FOR: REMEDIES FOR HEPATITIS

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 223313-1450

Declaration of Dr. Kazuko Hirabayashi Under 37 C.F.R. §1.132

I, Kazuko Hirabayashi, declare as follows:

1. I am a co-inventor of the present application.

2. I received my undergraduate degree from the Ochanomizu Women's Univerity in 1977 and my Ph.D. from the Tottori University of Medicine in 2000. I have been employed by Nippon Shinyaku since 1978 and I became a general manager of nucleic acids research and development in 2006. I have been a member of the American Association for Cancer Research since 2002.

3. I have read and understood US Pat. No. 5,298,614 ["Yano1"] and EP 0 685 457 A1["Yano 2"].

4. I have also read and understood Desmyter et al (Texas Reports on Biology and Medicine 35: 516-522 (1977) ["Desmyter"], Bever et al (Journal of Interferon Research 5: 423-428 (1985) ["Bever"], and Liaw (J. Gastroenterol. Hepatol. 1997, 12: S346-53).

5. At the time of the present invention, poly(I):poly(C) was known to induce interferon in mice and rabbits. [Black et al, Antimicrobial Agents and Chemotherapy, 3: 198-206

(1973); Tytell et al, PSEBM 135: 917-921 (1970)] Interferon has also been known to be effective for the treatment of hepatitis C at the time the present invention was made. However, it was recognized that poly(I):poly(C) can hardly be used as a medicine due to the fact that commercially available poly(I):poly(C) (unshortened poly(I):poly(C)) had a strong toxicity even though it could induce a sufficient plasma level of interferon and that shortened poly(I):poly(C) only weakly induced interferon in vivo.

6. My colleagues and I thought that it would be necessary to modify poly(I):poly(C) in various way in order to lower its toxicity with maintaining its efficacy so that it could be used as a medicine to treat hepatitis. At that time, however, we did not know how to modify poly(I):poly(C) to achieve this goal or even if it would be possible to achieve this goal.

7. We found for the first time that a specific combination of the shortened poly(I):poly(C), with a mean length within the range of 100 to 500 bp (100-500 bp poly(I):poly(C)), with a specific carrier comprising 2-O-(2-diethylaminoethyl)carbamoyl-1,3-O-dioleoylglycerol and a phospholipid induces a sufficient plasma concentration of interferon in vivo for treating hepatitis C with low toxicity. Moreover, this specific combination induces high, and probably even higher, concentrations of interferon in the liver, because it accumulates mainly in the liver. [See Specification at page 3, Figures 1 and 2]. Therefore, this specific combination of the present invention is useful for the treatment of hepatitis C.

8. My co-inventors and I have administered to a cynomolgus monkey an intravenous dose of a radioactive composition of the present invention, the 100-500 bp [^{3}H]poly(I):poly(C) complexed with LIC-101, which is a cationic liposome consisting

essentially of 2-O-(2-diethylaminoethyl) carbamoyl-1,3-O-dioleoylglycerol and lecithin phospholipid, and found that the complex was localized mainly in the liver, as shown in Table 1 below:

Table 1 . Localization of radioactivity (% of dose) in main tissues after an intravenous infusion of the 100-500 bp [^{3}H]poly(I):poly(C)/LIC-101 to a cynomolgus monkey (n = 1)

		Localization of radioactivity (% of dose)		
		0.083 h	1 h	4 h
[^{3}H-,^{14}C-] poly(I):poly(C)/ LIC-101 (^{3}H-Poly(C))	Plasma	50.5	4.9	1.9
	Heart	0.3	0.9	0.7
	[redacted]			
	Lung	1.2	0.6	0.7
	Kidney	1.8	4.5	5.4
	Spleen	2.5	2.1	1.3

Dose: 0.1 mg/kg [^{3}H]poly(I):poly(C)/LIC-101
LIC-101 is a cationic liposome consisting essentially of 2-O-(2-diethylaminoethyl) carbamoyl-1, 3-O-dioleoylglycerol and lecithin.
poly(I):poly(C)/LIC-101 means a complex of poly(I):poly(C) with LIC-101.

9. We also surprisingly found that intravenous administration of a low dose of 0.025 mg/kg [^{3}H]poly(I):poly(C)/LIC-101 to cynomolgus monkeys induced interferon-beta in the liver 3 hours after administration. See Table 2 below. In contrast, as described below, Desmyter, one of the reference cited by the Examiner, used 3 mg/kg of poly(I):poly(C) to treat hepatitis B.

Table 2. Induction of IFN-beta in Cynomolgus monkeys by [^{3}H]poly(I):poly(C)/LIC-101

	Serum	[redacted]
	IFN-beta (IU/ml)	
Control	n.d.	
Monkey number 1, 0.025 mg/kg	n.d.	
Monkey number 2, 0.025 mg/kg	n.d.	
Monkey number 3, 0.1 mg/kg	n.d.	

Monkey number 3, 0.1 mg/kg	n.d.	[redacted]

The 100-500 bp poly(I):poly(C)/LIC-101 was administered to Cynomolgus monkeys by intravenous injection. Three hours after the administration, the amount of IFN-beta in the liver and serum was measured by a human IFN-beta ELISA kit. The amount of IFN-beta was determined as IU/ml (Serum) or IU/g (Liver) in the table.
n.d.: not determined

10. We also found that shortened poly(I):poly(C) (50-600bp)/LIC-101 complex can induce the production of interferon-beta in HeLaS3 cells and that the length of the average chain length correlates with the production of interferon-beta as shown in Table 3:

Table 3. Induction of IFN-beta in HeLaS3 cells by poly(I):poly(C)/LIC-101

Average chane length of poly(I):poly(C) (bp)	Concentration of poly(I):poly(C)/LIC-101		
	1ng/ml	10ng/ml	100ng/ml
500- 600	0	36	190
300	0	34	130
150	0	20	92
80- 100	0	14	48
50- 60	0	12	36
20- 30	0	0	0

HeLaS3 cells were seeded at a density of 10^4 cells/well (96-well plate). The various length of poly(I):poly(C) and LIC-101 complexes were added to cells. Incubation was continued for 24 h. The amount of IFN-beta in the cell culture medium was measured by a human IFN-beta ELISA kit. The amount of IFN-beta was determined as IU/ml in the table.

11. It has also been shown that even though the 100-500 bp poly(I):poly(C) has low toxicity by itself (without being complexed with a cationic liposome and a phospholipid), it can not induce a sufficient plasma level of interferon in vivo for treating hepatitis.

12. In summary, the specific combination of the present invention can induce a clinically sufficient amount of interferon for the treatment of hepatitis C with very low toxicity.

When the specific combination is administered, long-lasting induction of interferon with the plasma level sufficient to treat hepatitis C occurs.

13. The above observations were not observed in any of the references cited by the Examiner. Because of the noted toxicities and high dosage used in the cited references, none of the references motivated my co-inventors or myself to come up with the idea of the claimed invention.

14. In Yano1, my colleague at Nippon Shinyaku Co. Ltd., Junichi Yano, describes how unmodified poly(I):poly(C) has an unexpectedly strong toxicity and problem in its administration to humans (see lines 41-50, in column 3 of Yano1). Therefore, the unmodified poly(I):poly(C) has been considered to be difficult to be developed as a medicine and had been neglected by my colleagues at Nippon Shinyaku Co. Ltd.

15. Yano 1 describes the use of a mismatched RNA (a part of -NH_2 groups substituted with –SH) as an interferon inducer. [Yano 1 at claims 1-3] Yano 1 does not even relate to poly(I):poly(C) and it does not disclose the cationic liposome of the present invention.

16. Yano 2 describes the cationic liposome of the type claimed in the specific composition of the present invention with a phospholipid, but it does not describe the specific combination of the cationic liposome of the present invention with poly(I):poly(C). Yano 2 makes no mention of the 100-500 bp poly I:polyC. Further, Yano 2 does not mention using the composition for treating hepatitis C.

17. Desmyter does not disclose administering poly(I):poly(C), let alone the 100-500 bp poly(I):poly(C), complexed with the cationic liposome consisting essentially of 2-O-(2-diethylaminoethyl) carbamoyl-1,3-O-dioleoylglycerol and a phospholipid.

18. Further, Desmyter uses a dose of 3 mg/kg, which is far higher than the claimed dose. Desmyter does not even relate to treating hepatitis C in the first instance. Rather, Desmyter only discusses hepatitis B. The fact that hepatitis B and hepatitis C are different in their responsiveness to interferon is beyond dispute. See the Liaw reference, cited by the Examiner, noting the differences between hepatitis B and hepatitis C, Abstract, Table 1.

19. Bever does not describe the use of poly(I):poly(C) , much less the 100-500 bp poly(I):poly(C) or a complex with the cationic liposome of the present invention. Bever does not mention the use of phospholipid and more importantly, Bever does not even relate to treating hepatitis; it relates to treating multiple sclerosis.

20. Similar to Bever, Liaw does not describe poly(I):poly(C) nor the 100-500 bp poly(I):poly(C), cationic liposomes, complexes of poly(I):poly(C) with cationic liposomes, or these complexes having phospholipid. Liaw describes therapeutic trends in therapy for chronic viral hepatitis, including hepatitis B (HBV) and C (HCV) infections and noted the difference between the two types of hepatitis in their responsiveness to interferon. Liaw noted that for HCV infection, interferon used at higher doses for a longer period of time is associated with a higher sustained response, but overall it is still not satisfactory. [Liaw Abstract]

21. It is my opinion that the composition of the claimed invention (having critical composition of poly(I):poly(C), with a mean length within the range of 100 to 500 bp, with a specific carrier comprising 2-O-(2-diethylaminoethyl)carbamoyl-1,3-O-dioleoylglycerol and a phospholipid) has surprising and unexpectedly superior interferon-

inducing properties with low toxicity when compared to the known compositions at the time the invention was made, and is thus useful for the treatment of hepatitis C.

22. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements so made are punishable by fine or imprisonment or both under § 1001 of Title 18 of the United States Code and that such willful statements may jeopardize the validity or enforceability of the present application or any patent issued thereon.

Kazuko Hirabayashi

Kazuko Hirabayashi

September 6, 2006

Date

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